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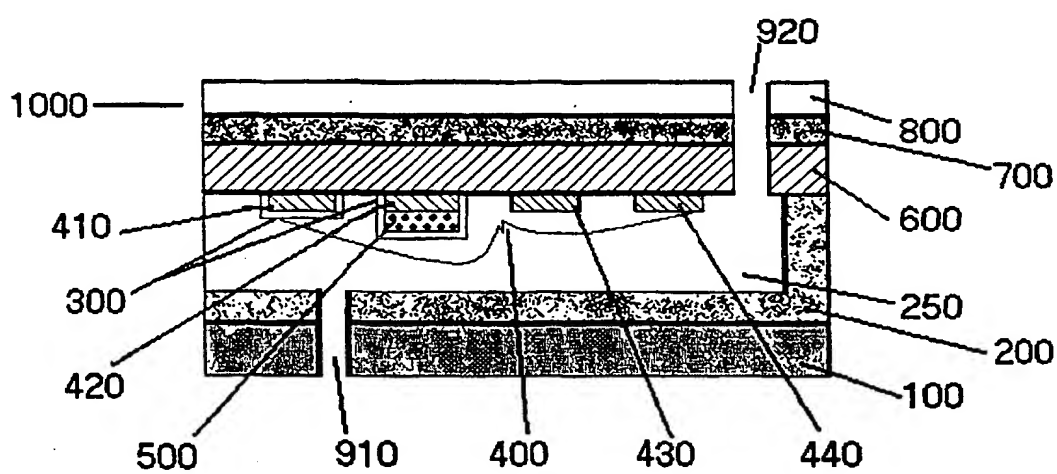

(57) Abstract

The present invention is directed at an assay device for detecting and enabling measurement of an analyte in a fluid. The assay device contains: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

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ASSAY DEVICE FOR MEASURING CHARACTERISTICS OF A FLUID ON A CONTINUAL BASIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Applications Serial No. 60/128,198 filed April 7, 1999; Serial No. 60/139,975 filed June 18, 1999; Serial No. 60/139,976 filed June 18, 1999; Serial No. 60/165,809 filed November 16, 1999; and Serial No. 60/182,698 filed February 15, 2000, all of which are incorporated by reference.

FIELD OF INVENTION

The present invention relates in general to analyte detection systems and methods. More specifically, this invention relates to an assay device that detects the presence, amount or other characteristic of an analyte of interest such as a glucose level within a fluid collected from a tissue on a continuous/continual basis.

BACKGROUND OF THE INVENTION

Medical studies have shown that the serious complications of diabetes can be significantly reduced by the proper control of the blood glucose levels. As a result, millions of diabetics monitor their blood glucose level on a daily basis via the traditional method of finger pricks and placing a blood sample into a testing apparatus. Some diabetics must monitor their blood glucose level more than just once a day. These individuals would greatly benefit from a system that continuously monitors blood glucose level without multiple finger pricks.

Attempts have been made to simplify the testing process and eliminate the need for blood. One method has been to illuminate the skin of the individual to determine the glucose level. Unfortunately, these attempts have failed to produce a viable product for continuous monitoring of blood glucose levels.

Another system under investigation is disclosed in U. S. Patent No. 5,961,451 to Reber et al. This patent sets forth a system which monitors the glucose level in the patient's interstitial fluid via an electrochemical assay device. However, this system is for one-time use only. The assay device must be replaced after every use. Similarly, U. S Patent No. 5,391,250 to Cheney II et al. and U. S. Patent No. 5,437,999 to Diebold et al. teach methods for fabricating electrochemical devices for one-time use biological applications.

Existing electrochemical testing systems have certain drawbacks to the individual user as these systems are usually expensive and inaccurate. In addition, these systems often have difficulties detecting low levels of analyte present in the interstitial fluid. Also, many of the previous systems are far too large for the individual user to use on a regular or continuous basis throughout the day.

Therefore, it would be advantageous to develop an analyte assay device that is useful to continuously monitor blood glucose levels.

SUMMARY OF THE INVENTION

The present invention relates to an assay device for detecting an analyte in a fluid, comprising: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well to discharge fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts

with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant. The membrane serves to extend the useful life of the assay device by slowing consumption of the reactant. As a result, the assay device is well suited for continuous monitoring applications.

In addition, the present invention relates to an assay device for detecting and enabling measurement of an analyte in a fluid comprising: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well to discharge fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) calibration port that is in fluid communication with the well.

Advantages of the invention will be obvious from the description, or may be learned by practice of the invention. Additional advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory of preferred embodiments of the invention, and are not restrictive of the invention, as claimed.

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate preferred and alternate embodiments of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross-section view of a preferred embodiment of the assay device according to the present invention.

Figure 2 is a cross-section view of an alternative embodiment of the assay device.

Figure 3 is a cross-section view of an another alternative embodiment of the assay device.

Figure 4 depicts an exploded view of still another embodiment of an assay device according to the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following figures and their previous and following description, including the detailed description of the invention and the examples provided herein. It is to be understood that this invention is not limited to the specific devices and methods described, as specific device components and/or process conditions as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must also be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" comprise plural referents unless the context clearly dictates otherwise. For example, reference to a component in the singular is intended to comprise a plurality of components.

As used herein, "analyte" shall mean the component that is being detected or measured in an analysis. In particular, the analyte may be any chemical or biological material or compound suitable for passage through a biological membrane technology known in the art, of which an individual might want to know the concentration or activity inside the body. Glucose is a specific example of an analyte because it is a sugar suitable for passage through the skin, and individuals, for example those having diabetes, might want to know their blood glucose levels. Other examples of analytes include, but are not limited to, such compounds as sodium, potassium, billirubin, urea, ammonia, calcium, lead, iron, lithium, salicylates, pharmaceutical compounds, and the like.

Ranges may be expressed herein as from "about" or "approximately" one particular value and/or to "about" or "approximately" another particular value. When such a range is expressed, another embodiment comprises from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment.

The present invention is directed at an assay device for detecting and enabling measurement of an analyte in a fluid. The assay device contains: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

Moreover, the present invention is directed to an assay device containing: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) calibration port that is in fluid communication with the well.

The assay device according to the present invention is suitable for use in a continuous/continual analyte monitoring system, such as that disclosed in International Application No. PCT/US99/16378, entitled "System and Method for Continuous Analyte Monitoring," filed July 20, 1999, which is incorporated herein by reference.

Referring now to **Figure 1**, a preferred embodiment of the assay device **1000** according to the present invention is shown. In this embodiment, the assay device **1000** comprises a bottom layer **100** that is in fluid communication with a channel-forming adhesive layer **200** via an inlet port **910**. The channel-forming adhesive layer **200** has a layer of adhesive with a channel cut into it to form a well **250**. Within the well **250**, are the membrane **300** and electrodes **400**.

In the preferred embodiment shown in **Figure 1**, there are four electrodes **400**: electrode **410** is a working electrode, electrode **420** is a working electrode, electrode **430** is a reference electrode and electrode **440** is a counter-electrode. At least one of the working electrodes **420** is coated with a reactant **500**. The electrodes **400** are disposed on or in a support base **600**. The support base **600** is adjacent to an adhesive layer **700**. An outlet port **920** extends through the support base layer **600**, the adhesive layer **700**, and a top layer **800**.

The bottom layer **100** provides structural support to the assay device **1000** and serves as the interface between the fluid source and the assay device **1000**. Any suitable material, in any thickness or shape may be used for the bottom layer **100**. Example suitable materials include acrylic, polyester, plastic, ceramic, polycarbonate and polyvinylchloride.

The inlet port **910**, which provides fluid communication between the bottom layer **100**, the channel-forming adhesive layer **200**, and the well **250**, may be in any position and in any dimension/shape to allow sufficient flow to the electrodes **400**. The inlet port **910** is suitable for alignment with holes/porations in a tissue from which fluid is to be drawn, such as interstitial fluid. An example of a mechanism to facilitate alignment of the assay device **1000** with the holes/porations in the tissue is disclosed in U.S. Provisional Application Serial No. 60/140,257 filed June 18, 1999 entitled "System and Method for Alignment of Micropores for Efficient Fluid Extraction and Substance Delivery," which is incorporated herein by reference.

The channel-forming adhesive layer **200** forms the well **250** to limit the volume of fluid within the assay device **1000**. Suitable materials for the channel-forming adhesive layer **200** are compatible with the fluid of interest, provide adhesive support to the assay device **1000**, and are thick enough to provide a well **250** from a channel cut into the channel-forming adhesive layer **200**. Preferably, the fluid of interest is blood or interstitial fluid, thereby requiring the channel-forming adhesive layer **200** to be constructed from adhesive-like materials that are not water-soluble.

The electrodes **400** are disposed on or in a support base **600** using screen-printing, pad printing, sputter coating, photolithography or other suitable techniques, using known inks and dielectrics. The support base **600** may be of

any thickness effective to provide support and bind the electrodes **400**. One preferred embodiment includes a support base **600** of 10 mil thick transparent polyester. Other suitable materials may be used including ceramic, polycarbonate, and polyvinylchloride.

Moreover, an adhesive layer **700** and a top layer **800** provide additional support to the assay device **1000**. The adhesive layer **700** binds the support base **600** to the top layer **800**. The material of construction and dimension of the adhesive layer **700** is not critical to the present invention, thereby allowing any effective adhesive to be used. The top layer **800**, like the bottom layer **100**, provides structural support to the assay device **1000**. Preferably, the top layer **800** is constructed of the same material or compatible material as the bottom layer **100**.

An outlet port **920** allows discharge of the fluid from the well **250** through the support base **600**, adhesive layer **700**, and the top layer **800**. It may be in any position and in any dimension/shape to allow sufficient flow to the electrodes **400**. The outlet port **920** also is suitable for connection to a supply of vacuum sufficient to draw fluid through the well **250**. In one preferred embodiment, the vacuum is sufficient to produce fluid from the skin at a site of where small holes/porations have been made in the tissue. The well **250** serves to expose the membrane **300** and electrodes **400** to the fluid that is monitored. Therefore, the well **250** is preferably of a dimension that the membrane **300** and the electrodes **400** do not obstruct the flow of fluid.

As explained in one example hereinafter, a reactant **500** reacts with the analyte to form a reaction product. The reaction product is in fluid communication with one or both of the working electrodes **410** and **420** whereby electrons are created. Depending on the reactant's composition, the reactant **500** may react with glucose, which may in turn form hydrogen peroxide. In this

embodiment, when hydrogen peroxide contacts a working electrode, oxygen gas, hydrogen ions and electrons are produced.

Each working electrode **410** and **420** may be made from a variety of materials such as carbon and metals such as gold or silver. Preferably, each working electrode **410** and **420** is made from catalytic metals such as platinum, palladium, chromium, ruthenium, rubidium, or mixtures thereof. Most preferably, the working electrodes **410** and **420** contain platinum.

To detect and/or measure the level of an analyte present in a fluid, at least one working electrode and at least one reference electrode are necessary. However, more than one working electrode and one or more counter-electrodes may also be present. For example, in the embodiment shown in **Figure 1**, the working electrode **410** does not contain the reactant and therefore it produces an electrical signal that is indicative of the fluid without the analyte. This allows reduction or elimination of the signal due to various interferent compounds by subtracting the electrical signal of the working electrode **410** from the electrical signal of the working electrode **420**.

Alternatively, one working electrode may be used if the levels of interference are not significant or if an interference blocking layer is included. This interference blocking layer could be positioned anywhere between the fluid to be analyzed and the working electrodes **410** and **420**. In one preferred embodiment, the interference blocking layer is placed directly over the working electrodes **410** and **420**. In another preferred embodiment, the interference blocking layer is placed adjacent to the membrane **300**. Suitable interference blocking layers include NAFION™ and cellulose acetate. Possible interferents include: acetaminophen, ascorbic acid, unconjugated bilirubin-, cholesterol, creatinine, dopamine, gentisic acid, heparin, ibuprofen, salicylate, tetracycline, tolbutamide, triglycerides and uric acid.

The reference electrode **430** establishes a potential relative to the fluid. Preferably, the reference electrode **430** contains silver/silver-chloride. The counter-electrode **440**, which is optional, serves to ground the current generated by the working electrodes **410** and **420**. Preferably, the counter-electrode **440** contains substantially the same materials as the working electrodes **410** and **420**. The assay device **1000** may contain more than one working electrode, more than one reference electrode and more than one counter-electrode, as is well known in the art.

The active surface of the electrodes **400** may be any shape and dimension to effectively operate. Particularly, the surface area of the any of the electrodes **400** can be varied as long as there is sufficient sensitivity to measuring the current. Preferably, the electrodes **400** have active surface areas between 0.1 mm^2 and 10 mm^2. Most preferably, the electrodes **400** have a surface area of 1mm^2.

After the assay device **1000** is constructed, the working electrodes **410** and **420** may be pre-conditioned by running at a specific voltage, such as +1.6 V relative to the reference electrode **430** for a suitable amount of time, such as 30 minutes, in a buffer system. This conditions the surface of the working electrodes **410** and **420** and increases their sensitivity to the reaction product generated by the reactant **500**. Alternately, the working electrodes **410** and **420** could be conditioned for shorter times at higher voltages.

The working potential will depend on the composition and shape of catalytic surface area. As such, the working potential can vary from 200 mV to 2 V. Such potential may be supplied via a monitoring unit coupled to the assay device wherein the monitoring unit utilizes an amperometric or coulometric measurement technique, known in the art. The working potential is generated

either by holding the working electrodes **410** and **420** at a positive potential or by holding the counter-electrode **440** at a negative potential. For example, the working electrodes **410** and **420** may be held at +800 mV and the reference electrode **430** and counter-electrode **440** at 0 mV, or the working electrodes **410** and **420** may be held at 0 mV and the reference electrode **430** and counter-electrode **440** at –800 mV.

The electrodes may be connected to leads that in turn are connected to a monitoring unit (**Figure 4**), patient worn or otherwise, via traces of graphite or silver/silver-chloride. However, other conductive material such as gold or tin are suitable to connect the electrodes to the leads. These traces could be applied via any method that provides a sufficient resolution such as ink-jet printing or pad printing. In addition, the printed traces could be replaced with traditional connection techniques.

A quantity of reactant **500** that reacts with the analyte to form a reaction product is disposed proximate to the at least one first working electrode such that when the analyte contacts the reactant **500**, the reaction product is in fluid communication with the working electrode. Preferably, the quantity of reactant **500** covers a portion of the first working electrode (working electrode **420** shown in **Figure 1**). The quantity of reactant **500** may also be disposed on or in at least one working electrode. The reactant **500** is selected to react with a specific analyte. In one preferred embodiment, the quantity of reactant **500** is suitable to react with glucose. As such, suitable reactants for the analyte glucose include glucose oxidase enzyme ("GOX"), glucose dehydrogenase ("GDH"), or mixtures thereof.

When the reactant **500** is chosen from this group, the glucose in the fluid makes contact with the reactant(s) to produce reaction products, which in the case of GOX, are gluconolactone and hydrogen peroxide. The hydrogen peroxide

diffuses to the working electrode **420** and reacts with the catalytic metal to produce electrons as described above. Alternatively, the reactants may include a mediator as an electron receptor instead of using oxygen. In such an embodiment, the mediator reacts with the working electrode **420** to produce electrons. Mediators that are commonly used are ferrocene, ferrocyamide and their derivatives.

In one preferred embodiment, the reactant **500** is prepared by mixing 8 mg/mL GOX with 60 mg/mL bovine serum albumen ("BSA") that is dissolved in phosphate buffered saline ("PBS") that contains 10% glycerol and 0.01% NaN_3. In this preferred embodiment, 20 μL of 25% glutaraldehyde is added to the mixture immediately before application. Preferably, a 1μL drop of this mixture is placed onto one of the working electrodes. The mixture is then allowed to solidify and cure at room temperature for approximately eight to sixteen hours.

The BSA serves as a carrier for the GOX due to its multiple cross-linking sites. As such, it can be replaced with any material that has multiple surface amine groups. In addition, the combination of BSA and glutaraldehyde as a cross linking system can be replaced with a system that will immoblize the active enzyme (in this case, GOX or GDH) without inhibiting its activity. Suitable replacements include other cross-linkers, polymer films, avidin-biotin linkages, antibody linkages, and covalent attachment to colloidal gold or agarose beads.

In this preferred embodiment, the PBS acts to maintain the reactant in a neutral pH range (such as a pH of about 6.5 to about 7.5). Any suitable buffer may be used. Example buffers include phosphate, citrate, Tris-HCl, MOPS, HEPES, MES, Bis-Tris, BES, ADA, ACES, MDPSO, Bis-Tris Propane, and TES. The glycerol serves to prevent the reactant **500** from becoming dehydrated which reduces the wetting time for later use. To this end, any suitable additive

may be used. The NaN_3 acts as an anti-bacterial agent. The NaN_3 could be replaced by any anti-microbial agent including antibiotics and detergents.

The glutaraldehyde is a cross-linking agent that links the GOX and BSA into a matrix that will not dissolve or move from the electrode surface. In one preferred embodiment, the glycerol could be present in an amount of 5% to 50% (by weight). Alternatively, the glycerol can be replaced or supplemented by any hygroscopic preservative or wetting agents including mild detergents such as TWEEN-20™, SPAN™, TRITON™, BRIJ™, MYRJ™ and PLURONICS™ familes of detergents.

The proportions of any of the components to the reactant **500** and the overall amount of reactant **500** are not critical to the present invention as long as the amount is effective. These proportions and overall amount of reactant **500** are limited by the preference of having an excess of reactant **500** as well as maintaining the reactant's solubility in the available volume. Preferably, the concentration of the reactant **500** is a minimum of 1 mg/mL.

The reactant **500** may be applied to a working electrode with any method that allows for volume and position control capable with techniques such as screen printing, ink-jet printing, air brush, and pad printing. For example, for application methods that use a nozzle or a screen that must continuously pass solution, the reactant **500** is preferably applied without glutaraldehyde, and then, the glutaraldehyde is placed down. This avoids fouling the nozzle with solidifying material. Once applied, the reactant **500** is dried and cured with the times of each varying based upon the amount and thickness of reactant layer(s) and the composition. Suitable drying conditions include temperatures up to 150°C, controlled humidity, and cure times of 15 minutes to 24 hours.

The GOX enzyme will saturate at concentrations of approximately 3 mM glucose. In order to detect higher levels of glucose, the concentration reaching the reactant **500** must be held to a fraction of the total concentration. To accomplish this, a membrane **300** is disposed over or around the reactant **500**. In one preferred embodiment, the membrane **300** is disposed over or around all of the electrodes **400** as shown in **Figure 3** In another preferred embodiment, the membrane **300** is disposed over or around the working electrodes **410** and **420** as shown in **Figure 2**. Alternatively, the membrane **300** is disposed over or around each electrode **400** or each working electrode **410** and **420** as shown in **Figures 1**.

The membrane **300** preferably is a diffusion-limiting membrane that extends the linear range and the lifetime of the assay device **1000** system and makes it useful in a continuous/continual monitoring system. The membrane **300** has pores which regulate diffusion of an analyte therethrough. Therefore, the membrane **300** may be sized to limit the rate at which the analyte or an interferent makes contact with the reactant **500,** thereby increasing the linear range of the assay device **1000**. For example, the membrane **300** may have low porosity to reduce glucose flux. As such, the membrane **300** limits the amount of analyte that is present at the electrodes at any one time, allowing the electrodes **400** to operate continuously over long periods of time without depleting the reactant. In fact, a monitoring unit coupled to the at least one working electrode may continuously draw fluid through the assay device and detect the presence or level of an analyte in excess of 24 hours, more preferably in excess of 48 hours, and still more preferably in excess of 70 hours.

One preferred membrane **300** is a 0.01 μm pore diameter polycarbonate ("PC") track-etch member 6 μm thick. Other suitable membranes that effectively produce a diffusion rate include dialysis membranes, polyurethane membranes, or polyvinylchloride membranes. Castable membranes such as NAFION™,

cellulose acetate, silastics and alkoxy silanes are also effective for this use. Additionally, multiple membranes may be used.

The membrane **300** may be secured by a layer of cross-linked BSA in the same buffer as the reactant **500**. In one preferred embodiment, the cross-linked BSA consists of 60 mg/mL BSA dissolved in PBS containing 10% glycerol and 0.01% NaN3. In addition, 20 μL/mL of 25% glutaraldehyde may be added immediately before use.

Any effective amount of this layer of cross-linked BSA may be used. In one preferred embodiment, a 2 μL drop of the cross-linked BSA is placed on the reactant covered electrode. Then, a 5 mm diameter circle of the 0.01 μm PC membrane is placed over the drop of cross-linked BSA or other suitable, large polyamine. The membrane **300** is gently pressed into place under a sheet of parafilm. It is allowed to cure for 16 hours at room temperature under the parafilm, and then the parafilm is removed.

The membrane **300** may be disposed over or around the reactant **500** by any suitable method including lamination, gluing, pressing, rolling and stretching. Any such method should not be destroyed by the fluid to be collected and analyzed. However, if an additional component is added, such as glue or other adhesion material, the additional component should be permeable in the fluid, such as Nafion is permeable in an aqueous-based fluid. Other suitable adhesives include epoxies, UV curable adhesives, pressure sensitive adhesives and hydrogels such as HEMA.

In operation, the assay device **1000** is positioned on a tissue site overlying one or more openings made in the tissue. The openings in the tissue may be made by a variety of means, such as those disclosed in commonly assigned U.S. Patent No. 5,885,211. Fluid enters the assay device **1000** of **Figure 1** through the

inlet port **910**. Under application of vacuum at the outlet port **920**, the fluid travels through the well **250** and contacts the membrane **300**. The membrane **300** is permeable to the analyte in the fluid and thereby allows the analyte to contact the electrodes **400** and the reactant **500**. The analyte reacts with the reactant **500** to generate a reaction product. The reaction product contacts the working electrode **420** and creates electrons, thereby generating a current flow. The fluid continues through the outlet port **920** whereupon it exits the assay device **1000**. Current flow across the working electrode(s) is measured, and from this a measurement of the analyte is obtained. The assay device **1000** may be used in conjunction with amperometric and coulometric measuring techniques.

In an amperometric measurement the current (charge/second) is measured at the applied voltage. This can be measured continuously, which is a preferred method in a flowing system. With a coulometric technique, the total charge accumulated over a period of time is measured after a voltage is applied. Typically, the fluid is allowed to react with the reactant over a fixed period of time, thereby generating a reaction product. Then a voltage is applied and the current, which is measured over a fixed period of time, is integrated (added) to calculate the total amount of charge produced by the reaction product. This alternative method has the advantage of generating larger signals and reducing the impact of electroactive interfering substances.

The membrane **300** preserves the life of the reactant **500** by helping to hold the reactant **500** in place and to thereby reduce the risk of rapid dissolution of the reactant **500** in the fluid. Also, by restricting the amount of analyte and interferents to the reactant **500**, the membrane **300** helps ensure that the reactant **500** is in excess than what is needed to fully engage the analyte. In this way, as the reactant **500** degrades over time, it may remain in excess and deterioration in performance will be minimized.

Referring now to **Figure 2,** a variation of the assay device **1000** is shown in which a well **250** opens into an outlet port **920**. In such a configuration, the outlet port **920** does not provide fluid communication through the support base **600**, the adhesive layer **700**, and the top layer **800**. However, the outlet port **920** is suitable for connection to a supply of vacuum sufficient to draw fluid through the well **250**. The outlet port **920** may be filled with wiring and drain tubing, and then epoxy sealed.

Figure 3 shows another alternative preferred embodiment of the assay device **1000**. As in **Figure 2**, the well **250** is coupled to the outlet port **920**. However, **Figure 3** shows an assay device that comprises only a support base **600** and bottom layer **100.** The bottom layer **100** provides the well **250** to expose the membrane **300** and electrodes **400** to the fluid.

Figure 4 shows another embodiment of an assay device **1000** according to the present invention. This embodiment includes a bottom layer **100**, a channel-forming adhesive layer **200,** a support base **600**, an adhesive layer **700**, and a top layer **800**. **Figure 4** also includes an inlet port **910**, a well **250**, an outlet port **920**, a calibration port **950**, and drain tubing **940**. Although not shown, this embodiment includes at least one working electrode, a reference electrode, and a reactant proximate to the working electrode as shown in **Figures 1-3**. This embodiment may optionally include at least one membrane, as shown in **Figure 1**. The electrodes are connected to a monitoring unit **970** via leads **960**. The monitoring unit **970** is also connected to assay device **1000** via the drain tubing **940**. The drain tubing **940** provides vacuum to the assay device **1000**.

Similar to the assay device **1000** of **Figure 2,** the well **250** shown in **Figure 4** opens into the outlet port **920**. The calibration port **950** is suitable for connection to a reservoir **980** containing calibration fluid. In addition, the

calibration port **950** may include a membrane **990** permeable to the calibration fluid.

In one embodiment, the calibration fluid consists of water and the analyte to be detected. Other compounds may also be present, such as surfactants, which ensure smoother flow by reducing surface tension, such as SDS, or any of the detergents herein described. Additionally, preservatives such as azide, EDTA, or any antibacterial or appropriate biocide that will not degrade of interfere with the reactant's performance may be added to the calibration fluid. Moreover, the calibration fluid may include thickeners, such as polymers and proteins, to simulate the flow characteristics of the analyte-containing fluid that is being measured.

The reservoir **980** is in fluid communication with the well **250** such that the calibration fluid flushes the well **250** to contact the electrodes with the calibration fluid. The calibration fluid is removed from the well **250** through the outlet port **920** under application of vacuum.

The reservoir **980** may release the calibration fluid into the well **250** using any effective mechanism. In one embodiment, the reservoir **980** comprises a bag-like member that opens and releases the calibration fluid into the well **250** in response to application of vacuum applied at the outlet port **920**. Moreover, the reservoir **980** may be formed of a material that when mechanically punctured releases the calibration fluid into the well **250**.

Alternatively, the membrane **990** is a self-sealing membrane that is rupturable to allow introduction of the calibration fluid such as by a syringe containing calibration fluid to deliver the calibration fluid into the well **250**. Preferably, the calibration fluid is delivered into the well **250** while vacuum is applied at the outlet port **920**. In another preferred embodiment, the calibration

fluid is introduced into the well **250** via a valve that operates as a one-way valve or is controlled external to the assay device **1000**.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. An assay device for detecting an analyte in a fluid, comprising:
   a) an inlet port to receive fluid;
   b) a well in fluid communication with the inlet port;
   c) an outlet port in fluid communication with the well to discharge fluid;
   d) at least one first working electrode and at least one reference electrode disposed within the well;
   e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and
   f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

2. The assay device of claim 1, wherein the inlet port is suitable for alignment with holes/porations in tissue from which fluid is drawn.

3. The assay device of claim 1, wherein the outlet port is suitable for connection to a supply of vacuum sufficient to draw fluid through the well.

4. The assay device of claim 1, wherein the at least one first working electrode is comprised of a catalytic metal.

5. The assay device of claim 1, wherein the at least one first working electrode is comprised of platinum, palladium, chromium, ruthenium, rubidium, or mixtures thereof.

6. The assay device of claim 1, wherein the at least one reference electrode is comprised of silver/silver-chloride.

7. The assay device of claim 1, further comprising at least one counter-electrode disposed within the well.

8. The assay device of claim 1, further comprising a second working electrode disposed within the well.

9. The assay device of claim 1, further comprising at least one counter-electrode and a second working electrode disposed within the well.

10. A monitoring system comprising the assay device of claim 1, and further comprising a monitoring unit coupled to the assay device.

11. A monitoring system comprising the assay device of claim 1, and further comprising a monitoring unit coupled to the assay device, wherein the monitoring unit generates an analyte measurement from the assay device utilizing a coulometric or amperometric measurement technique.

12. The assay device of claim 1, wherein the quantity of reactant is disposed in or on the at least one working electrode.

13. The assay device of claim 1, wherein the quantity of reactant is comprised of glucose oxidase enzyme, glucose dehydrogenase, or mixtures thereof.

14. The assay device of claim 1, wherein the quantity of reactant is suitable to react with glucose.

15. The assay device of claim 1, wherein the at least one membrane comprises pores which are sized to limit the rate at which the analyte makes contact with the reactant.

16. The assay device of claim 1, wherein the at least one membrane comprises pores which are sized to limit the rate at which an interferent makes contact with the reactant.

17. The assay device of claim 1, wherein the at least one membrane is disposed over or around the at least one first working electrode.

18. The assay device of claim 1, wherein the at least one membrane is disposed over or around the at least one first working electrode and the at least one reference electrode.

19. The assay device of claim 1, further comprising a second working electrode disposed within the well and wherein the at least one membrane is disposed over or around the at least one first working electrode and the second working electrode.

20. The assay device of claim 1, further comprising a reservoir containing a calibration fluid, wherein the reservoir is in fluid communication with the well such that the calibration fluid flushes the well and is removed from the well through the outlet port.

21. The assay device of claim 20, wherein the reservoir comprises a bag that opens and releases the calibration fluid into the well in response to application of vacuum thereto applied at the outlet port.

22. The assay device of claim 20, wherein the reservoir is formed of a material that when mechanically punctured releases the calibration fluid into the well.

23. The assay device of claim 20, further comprising a calibration port that couples the reservoir to the well, wherein the calibration port comprises a membrane permeable to the calibration fluid.

24. An assay device for detecting and enabling measurement of an analyte in a fluid, comprising:
  a) an inlet port to receive fluid,
  b) a well in fluid communication with the inlet port,
  c) an outlet port in fluid communication with the well to discharge fluid,
  d) at least one first working electrode and at least one reference electrode disposed within the well,
  e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and
  f) a calibration port that is in fluid communication with the well.

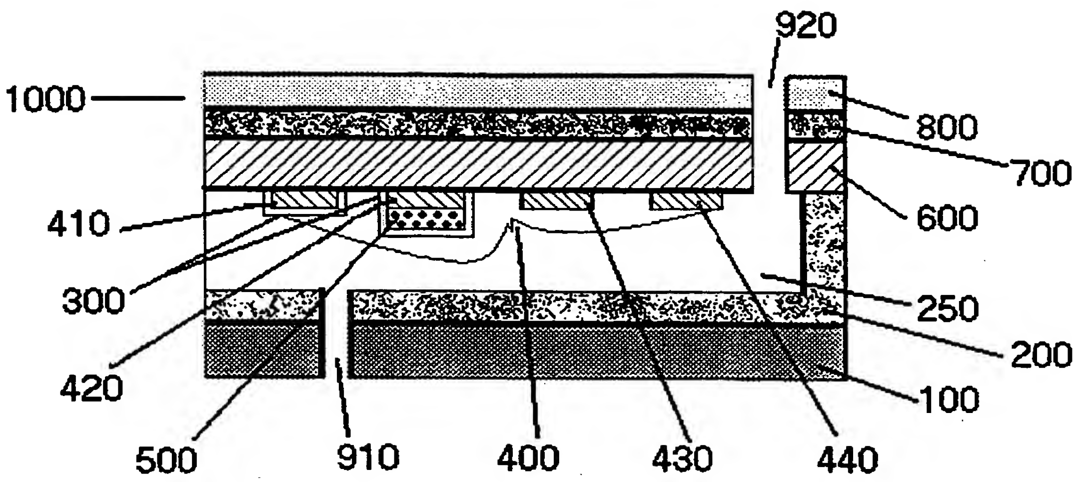

Figure 1

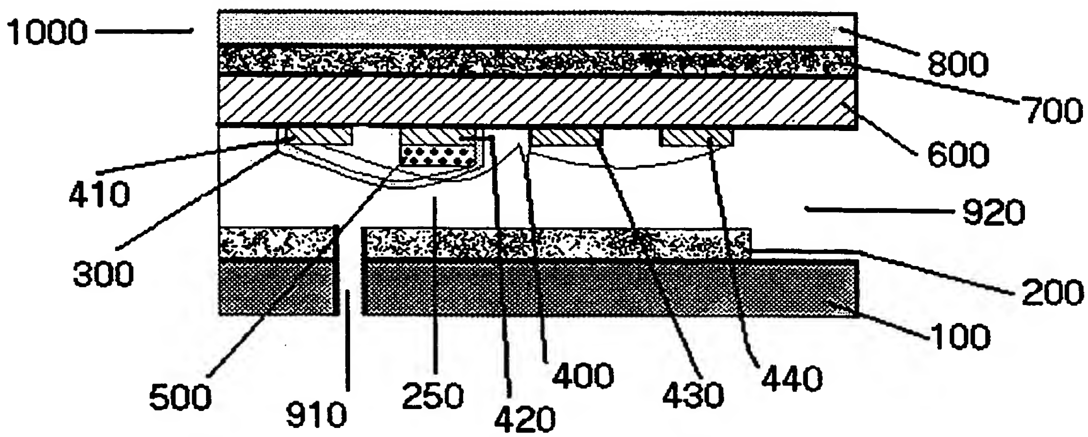

Figure 2

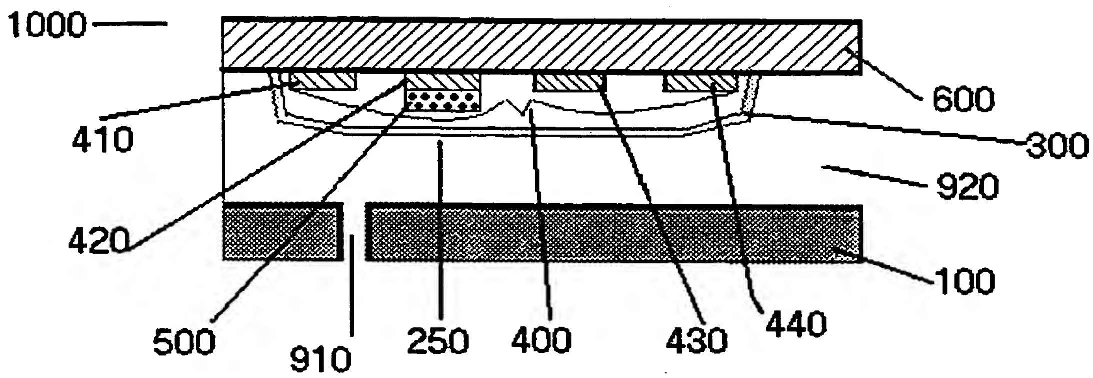

Figure 3

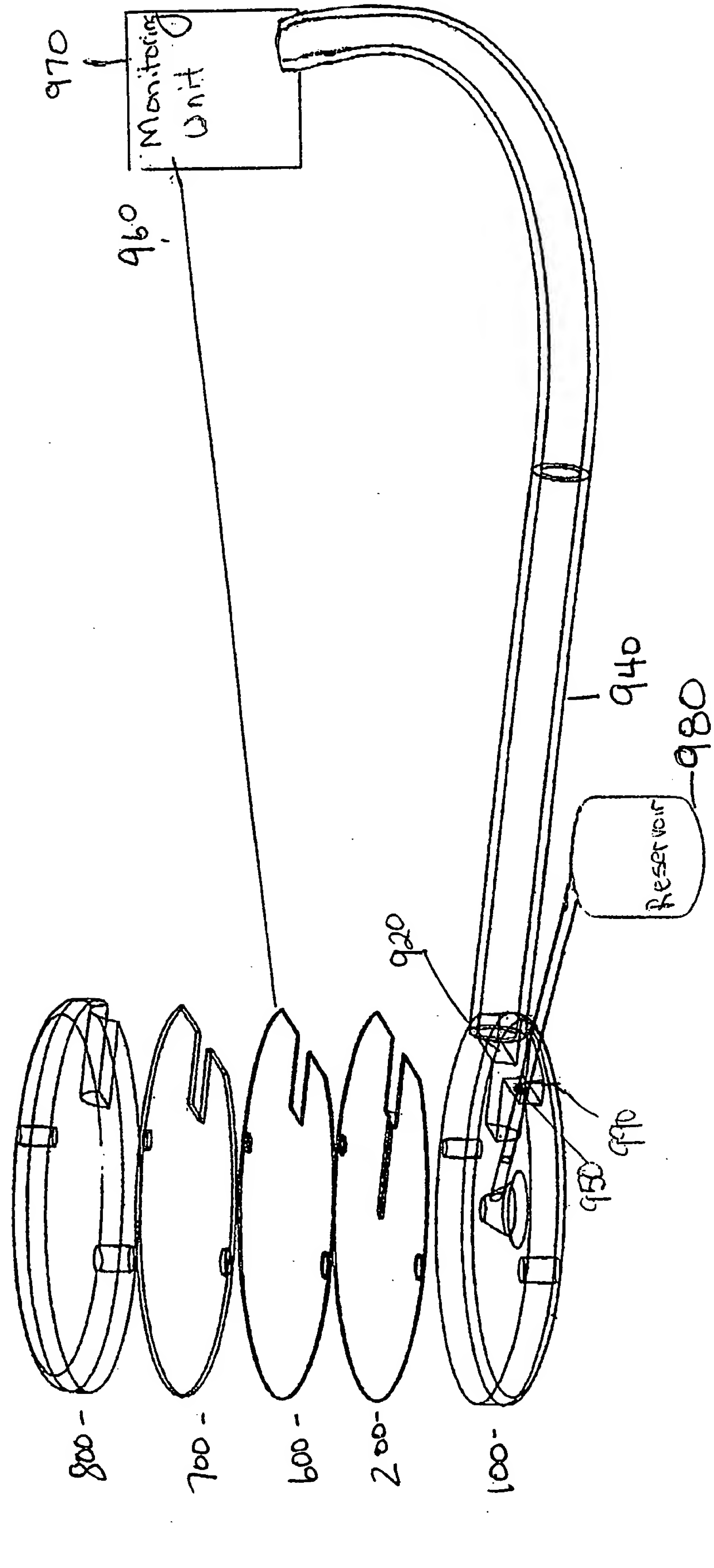
Monitoring Unit
970
960
940
980
Reservoir
920
910
950
800
700
600
200
100

Figure 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/09393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STEINKUHL R ET AL: "MICRODIALYSIS SYSTEM FOR CONTINUOUS GLUCOSE MONITORING" SENSORS AND ACTUATORS B,CH,ELSEVIER SEQUOIA S.A., LAUSANNE, vol. B33, no. 1/03, 1 July 1996 (1996-07-01), pages 19-24, XP000632919 ISSN: 0925-4005	1,4-6, 10-14,17
A	page 20, left-hand column, line 21 -page 21, left-hand column, line 43; table 1	2,15,16, 24

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2000	27/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Weihs, J

1

International Application No
PCT/US 00/09393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HINTSCHE R ET AL: "CHEMICAL MICROSENSOR SYSTEMS FOR MEDICAL APPLICATIONS IN CATHETERS" SENSORS AND ACTUATORS B,CH,ELSEVIER SEQUOIA S.A., LAUSANNE, vol. B27, no. 1/03, PART 02, 1 June 1995 (1995-06-01), pages 471-473, XP000516364 ISSN: 0925-4005	1,3-6, 11-14,17
A	page 471, right-hand column, line 1 -page 473, left-hand column, line 2; tables 1,2	15,16,24
Y	EP 0 453 283 A (TEKNEKRON SENSOR DEV CORP) 23 October 1991 (1991-10-23) column 2, line 18 -column 4, line 22; tables 1-3	1-7, 10-18,24
Y	WO 98 30891 A (IMPLANTED BIOSYSTEMS INC) 16 July 1998 (1998-07-16)	1-7, 10-18,24
A	page 4, line 27 -page 12, line 2; tables 2-4	20-23

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/09393

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0453283	A	23-10-1991	US	5161532 A	10-11-1992
			JP	4227235 A	17-08-1992
WO 9830891	A	16-07-1998	US	5914026 A	22-06-1999
			AU	5904598 A	03-08-1998

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60/165,809	16 November 1999 (16.11.1999)	US
60/182,698	15 February 2000 (15.02.2000)	US

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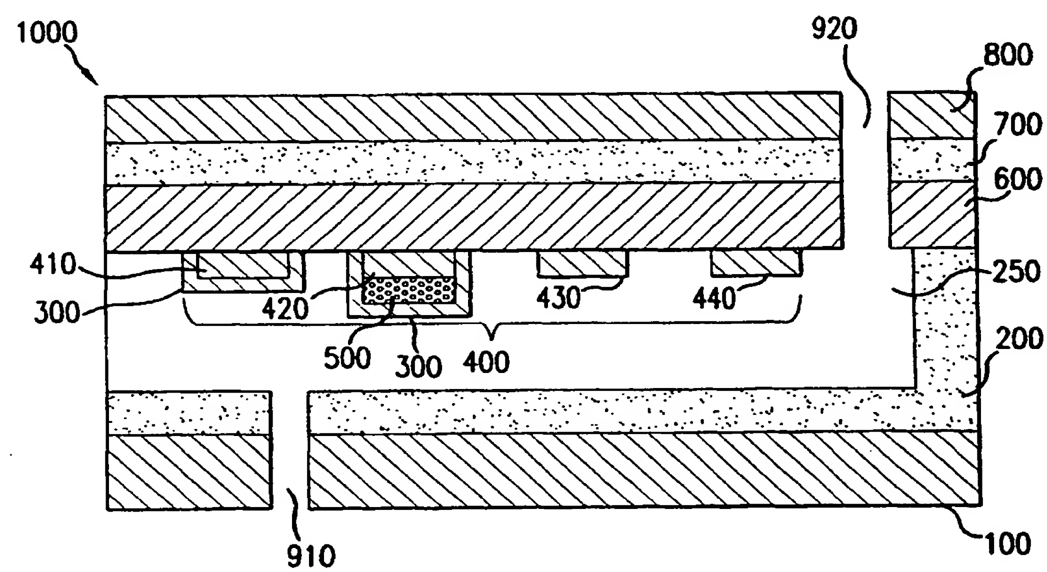
(74) Agents: **FLOAM, D., Andrew** et al.; Needle & Rosenberg, P.C., The Candler Building, Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303-1811 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,

[Continued on next page]

(54) Title: ASSAY DEVICE FOR MEASURING CHARACTERISTICS OF A FLUID ON A CONTINUAL BASIS



(57) Abstract: The present invention is directed at an assay device for detecting and enabling measurement of an analyte in a fluid. The assay device contains: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

WO 00/59373 A1

DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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ASSAY DEVICE FOR MEASURING CHARACTERISTICS OF A FLUID ON A CONTINUAL BASIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Applications Serial No. 60/128,198 filed April 7, 1999; Serial No. 60/139,975 filed June 18, 1999; Serial No. 60/139,976 filed June 18, 1999; Serial No. 60/165,809 filed November 16, 1999; and Serial No. 60/182,698 filed February 15, 2000, all of which are incorporated by reference.

FIELD OF INVENTION

The present invention relates in general to analyte detection systems and methods. More specifically, this invention relates to an assay device that detects the presence, amount or other characteristic of an analyte of interest such as a glucose level within a fluid collected from a tissue on a continuous/continual basis.

BACKGROUND OF THE INVENTION

Medical studies have shown that the serious complications of diabetes can be significantly reduced by the proper control of the blood glucose levels. As a result, millions of diabetics monitor their blood glucose level on a daily basis via the traditional method of finger pricks and placing a blood sample into a testing apparatus. Some diabetics must monitor their blood glucose level more than just once a day. These individuals would greatly benefit from a system that continuously monitors blood glucose level without multiple finger pricks.

Attempts have been made to simplify the testing process and eliminate the need for blood. One method has been to illuminate the skin of the individual to determine the glucose level. Unfortunately, these attempts have failed to produce a viable product for continuous monitoring of blood glucose levels.

Another system under investigation is disclosed in U. S. Patent No. 5,961,451 to Reber et al. This patent sets forth a system which monitors the glucose level in the patient's interstitial fluid via an electrochemical assay device. However, this system is for one-time use only. The assay device must be replaced after every use. Similarly, U. S Patent No. 5,391,250 to Cheney II et al. and U. S. Patent No. 5,437,999 to Diebold et al. teach methods for fabricating electrochemical devices for one-time use biological applications.

Existing electrochemical testing systems have certain drawbacks to the individual user as these systems are usually expensive and inaccurate. In addition, these systems often have difficulties detecting low levels of analyte present in the interstitial fluid. Also, many of the previous systems are far too large for the individual user to use on a regular or continuous basis throughout the day.

Therefore, it would be advantageous to develop an analyte assay device that is useful to continuously monitor blood glucose levels.

SUMMARY OF THE INVENTION

The present invention relates to an assay device for detecting an analyte in a fluid, comprising: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well to discharge fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts

with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant. The membrane serves to extend the useful life of the assay device by slowing consumption of the reactant. As a result, the assay device is well suited for continuous monitoring applications.

In addition, the present invention relates to an assay device for detecting and enabling measurement of an analyte in a fluid comprising: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well to discharge fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) calibration port that is in fluid communication with the well.

Advantages of the invention will be obvious from the description, or may be learned by practice of the invention. Additional advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory of preferred embodiments of the invention, and are not restrictive of the invention, as claimed.

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate preferred and alternate embodiments of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross-section view of a preferred embodiment of the assay device according to the present invention.

Figure 2 is a cross-section view of an alternative embodiment of the assay device.

Figure 3 is a cross-section view of an another alternative embodiment of the assay device.

Figure 4 depicts an exploded view of still another embodiment of an assay device according to the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following figures and their previous and following description, including the detailed description of the invention and the examples provided herein. It is to be understood that this invention is not limited to the specific devices and methods described, as specific device components and/or process conditions as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must also be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" comprise plural referents unless the context clearly dictates otherwise. For example, reference to a component in the singular is intended to comprise a plurality of components.

As used herein, "analyte" shall mean the component that is being detected or measured in an analysis. In particular, the analyte may be any chemical or biological material or compound suitable for passage through a biological membrane technology known in the art, of which an individual might want to know the concentration or activity inside the body. Glucose is a specific example of an analyte because it is a sugar suitable for passage through the skin, and individuals, for example those having diabetes, might want to know their blood glucose levels. Other examples of analytes include, but are not limited to, such compounds as sodium, potassium, billirubin, urea, ammonia, calcium, lead, iron, lithium, salicylates, pharmaceutical compounds, and the like.

Ranges may be expressed herein as from "about" or "approximately" one particular value and/or to "about" or "approximately" another particular value. When such a range is expressed, another embodiment comprises from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment.

The present invention is directed at an assay device for detecting and enabling measurement of an analyte in a fluid. The assay device contains: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

Moreover, the present invention is directed to an assay device containing: a) an inlet port to receive fluid; b) a well in fluid communication with the inlet port; c) an outlet port in fluid communication with the well, wherein the outlet port is designed to allow discharge of the fluid; d) at least one first working electrode and at least one reference electrode disposed within the well; e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and f) calibration port that is in fluid communication with the well.

The assay device according to the present invention is suitable for use in a continuous/continual analyte monitoring system, such as that disclosed in International Application No. PCT/US99/16378, entitled "System and Method for Continuous Analyte Monitoring," filed July 20, 1999, which is incorporated herein by reference.

Referring now to **Figure 1**, a preferred embodiment of the assay device **1000** according to the present invention is shown. In this embodiment, the assay device **1000** comprises a bottom layer **100** that is in fluid communication with a channel-forming adhesive layer **200** via an inlet port **910**. The channel-forming adhesive layer **200** has a layer of adhesive with a channel cut into it to form a well **250**. Within the well **250**, are the membrane **300** and electrodes **400**.

In the preferred embodiment shown in **Figure 1**, there are four electrodes **400**: electrode **410** is a working electrode, electrode **420** is a working electrode, electrode **430** is a reference electrode and electrode **440** is a counter-electrode. At least one of the working electrodes **420** is coated with a reactant **500**. The electrodes **400** are disposed on or in a support base **600**. The support base **600** is adjacent to an adhesive layer **700**. An outlet port **920** extends through the support base layer **600**, the adhesive layer **700**, and a top layer **800**.

The bottom layer **100** provides structural support to the assay device **1000** and serves as the interface between the fluid source and the assay device **1000**. Any suitable material, in any thickness or shape may be used for the bottom layer **100**. Example suitable materials include acrylic, polyester, plastic, ceramic, polycarbonate and polyvinylchloride.

The inlet port **910**, which provides fluid communication between the bottom layer **100**, the channel-forming adhesive layer **200**, and the well **250**, may be in any position and in any dimension/shape to allow sufficient flow to the electrodes **400**. The inlet port **910** is suitable for alignment with holes/porations in a tissue from which fluid is to be drawn, such as interstitial fluid. An example of a mechanism to facilitate alignment of the assay device **1000** with the holes/porations in the tissue is disclosed in U.S. Provisional Application Serial No. 60/140,257 filed June 18, 1999 entitled "System and Method for Alignment of Micropores for Efficient Fluid Extraction and Substance Delivery," which is incorporated herein by reference.

The channel-forming adhesive layer **200** forms the well **250** to limit the volume of fluid within the assay device **1000**. Suitable materials for the channel-forming adhesive layer **200** are compatible with the fluid of interest, provide adhesive support to the assay device **1000**, and are thick enough to provide a well **250** from a channel cut into the channel-forming adhesive layer **200**. Preferably, the fluid of interest is blood or interstitial fluid, thereby requiring the channel-forming adhesive layer **200** to be constructed from adhesive-like materials that are not water-soluble.

The electrodes **400** are disposed on or in a support base **600** using screen-printing, pad printing, sputter coating, photolithography or other suitable techniques, using known inks and dielectrics. The support base **600** may be of

any thickness effective to provide support and bind the electrodes **400**. One preferred embodiment includes a support base **600** of 10 mil thick transparent polyester. Other suitable materials may be used including ceramic, polycarbonate, and polyvinylchloride.

Moreover, an adhesive layer **700** and a top layer **800** provide additional support to the assay device **1000**. The adhesive layer **700** binds the support base **600** to the top layer **800**. The material of construction and dimension of the adhesive layer **700** is not critical to the present invention, thereby allowing any effective adhesive to be used. The top layer **800**, like the bottom layer **100**, provides structural support to the assay device **1000**. Preferably, the top layer **800** is constructed of the same material or compatible material as the bottom layer **100**.

An outlet port **920** allows discharge of the fluid from the well **250** through the support base **600**, adhesive layer **700**, and the top layer **800**. It may be in any position and in any dimension/shape to allow sufficient flow to the electrodes **400**. The outlet port **920** also is suitable for connection to a supply of vacuum sufficient to draw fluid through the well **250**. In one preferred embodiment, the vacuum is sufficient to produce fluid from the skin at a site of where small holes/porations have been made in the tissue. The well **250** serves to expose the membrane **300** and electrodes **400** to the fluid that is monitored. Therefore, the well **250** is preferably of a dimension that the membrane **300** and the electrodes **400** do not obstruct the flow of fluid.

As explained in one example hereinafter, a reactant **500** reacts with the analyte to form a reaction product. The reaction product is in fluid communication with one or both of the working electrodes **410** and **420** whereby electrons are created. Depending on the reactant's composition, the reactant **500** may react with glucose, which may in turn form hydrogen peroxide. In this

embodiment, when hydrogen peroxide contacts a working electrode, oxygen gas, hydrogen ions and electrons are produced.

Each working electrode **410** and **420** may be made from a variety of materials such as carbon and metals such as gold or silver. Preferably, each working electrode **410** and **420** is made from catalytic metals such as platinum, palladium, chromium, ruthenium, rubidium, or mixtures thereof. Most preferably, the working electrodes **410** and **420** contain platinum.

To detect and/or measure the level of an analyte present in a fluid, at least one working electrode and at least one reference electrode are necessary. However, more than one working electrode and one or more counter-electrodes may also be present. For example, in the embodiment shown in **Figure 1**, the working electrode **410** does not contain the reactant and therefore it produces an electrical signal that is indicative of the fluid without the analyte. This allows reduction or elimination of the signal due to various interferent compounds by subtracting the electrical signal of the working electrode **410** from the electrical signal of the working electrode **420**.

Alternatively, one working electrode may be used if the levels of interference are not significant or if an interference blocking layer is included. This interference blocking layer could be positioned anywhere between the fluid to be analyzed and the working electrodes **410** and **420**. In one preferred embodiment, the interference blocking layer is placed directly over the working electrodes **410** and **420**. In another preferred embodiment, the interference blocking layer is placed adjacent to the membrane **300**. Suitable interference blocking layers include NAFION™ and cellulose acetate. Possible interferents include: acetaminophen, ascorbic acid, unconjugated bilirubin-, cholesterol, creatinine, dopamine, gentisic acid, heparin, ibuprofen, salicylate, tetracycline, tolbutamide, triglycerides and uric acid.

The reference electrode **430** establishes a potential relative to the fluid. Preferably, the reference electrode **430** contains silver/silver-chloride. The counter-electrode **440**, which is optional, serves to ground the current generated by the working electrodes **410** and **420**. Preferably, the counter-electrode **440** contains substantially the same materials as the working electrodes **410** and **420**. The assay device **1000** may contain more than one working electrode, more than one reference electrode and more than one counter-electrode, as is well known in the art.

The active surface of the electrodes **400** may be any shape and dimension to effectively operate. Particularly, the surface area of the any of the electrodes **400** can be varied as long as there is sufficient sensitivity to measuring the current. Preferably, the electrodes **400** have active surface areas between 0.1 mm^2 and 10 mm^2. Most preferably, the electrodes **400** have a surface area of 1mm^2.

After the assay device **1000** is constructed, the working electrodes **410** and **420** may be pre-conditioned by running at a specific voltage, such as +1.6 V relative to the reference electrode **430** for a suitable amount of time, such as 30 minutes, in a buffer system. This conditions the surface of the working electrodes **410** and **420** and increases their sensitivity to the reaction product generated by the reactant **500**. Alternately, the working electrodes **410** and **420** could be conditioned for shorter times at higher voltages.

The working potential will depend on the composition and shape of catalytic surface area. As such, the working potential can vary from 200 mV to 2 V. Such potential may be supplied via a monitoring unit coupled to the assay device wherein the monitoring unit utilizes an amperometric or coulometric measurement technique, known in the art. The working potential is generated

either by holding the working electrodes **410** and **420** at a positive potential or by holding the counter-electrode **440** at a negative potential. For example, the working electrodes **410** and **420** may be held at +800 mV and the reference electrode **430** and counter-electrode **440** at 0 mV, or the working electrodes **410** and **420** may be held at 0 mV and the reference electrode **430** and counter-electrode **440** at –800 mV.

The electrodes may be connected to leads that in turn are connected to a monitoring unit (**Figure 4**), patient worn or otherwise, via traces of graphite or silver/silver-chloride. However, other conductive material such as gold or tin are suitable to connect the electrodes to the leads. These traces could be applied via any method that provides a sufficient resolution such as ink-jet printing or pad printing. In addition, the printed traces could be replaced with traditional connection techniques.

A quantity of reactant **500** that reacts with the analyte to form a reaction product is disposed proximate to the at least one first working electrode such that when the analyte contacts the reactant **500**, the reaction product is in fluid communication with the working electrode. Preferably, the quantity of reactant **500** covers a portion of the first working electrode (working electrode **420** shown in **Figure 1**). The quantity of reactant **500** may also be disposed on or in at least one working electrode. The reactant **500** is selected to react with a specific analyte. In one preferred embodiment, the quantity of reactant **500** is suitable to react with glucose. As such, suitable reactants for the analyte glucose include glucose oxidase enzyme ("GOX"), glucose dehydrogenase ("GDH"), or mixtures thereof.

When the reactant **500** is chosen from this group, the glucose in the fluid makes contact with the reactant(s) to produce reaction products, which in the case of GOX, are gluconolactone and hydrogen peroxide. The hydrogen peroxide

diffuses to the working electrode **420** and reacts with the catalytic metal to produce electrons as described above. Alternatively, the reactants may include a mediator as an electron receptor instead of using oxygen. In such an embodiment, the mediator reacts with the working electrode **420** to produce electrons. Mediators that are commonly used are ferrocene, ferrocyamide and their derivatives.

In one preferred embodiment, the reactant **500** is prepared by mixing 8 mg/mL GOX with 60 mg/mL bovine serum albumen ("BSA") that is dissolved in phosphate buffered saline ("PBS") that contains 10% glycerol and 0.01% NaN_3. In this preferred embodiment, 20 μL of 25% glutaraldehyde is added to the mixture immediately before application. Preferably, a 1μL drop of this mixture is placed onto one of the working electrodes. The mixture is then allowed to solidify and cure at room temperature for approximately eight to sixteen hours.

The BSA serves as a carrier for the GOX due to its multiple cross-linking sites. As such, it can be replaced with any material that has multiple surface amine groups. In addition, the combination of BSA and glutaraldehyde as a cross linking system can be replaced with a system that will immoblize the active enzyme (in this case, GOX or GDH) without inhibiting its activity. Suitable replacements include other cross-linkers, polymer films, avidin-biotin linkages, antibody linkages, and covalent attachment to colloidal gold or agarose beads.

In this preferred embodiment, the PBS acts to maintain the reactant in a neutral pH range (such as a pH of about 6.5 to about 7.5). Any suitable buffer may be used. Example buffers include phosphate, citrate, Tris-HCl, MOPS, HEPES, MES, Bis-Tris, BES, ADA, ACES, MDPSO, Bis-Tris Propane, and TES. The glycerol serves to prevent the reactant **500** from becoming dehydrated which reduces the wetting time for later use. To this end, any suitable additive

may be used. The NaN_3 acts as an anti-bacterial agent. The NaN_3 could be replaced by any anti-microbial agent including antibiotics and detergents.

The glutaraldehyde is a cross-linking agent that links the GOX and BSA into a matrix that will not dissolve or move from the electrode surface. In one preferred embodiment, the glycerol could be present in an amount of 5% to 50% (by weight). Alternatively, the glycerol can be replaced or supplemented by any hygroscopic preservative or wetting agents including mild detergents such as TWEEN-20™, SPAN™, TRITON™, BRIJ™, MYRJ™ and PLURONICS™ familes of detergents.

The proportions of any of the components to the reactant **500** and the overall amount of reactant **500** are not critical to the present invention as long as the amount is effective. These proportions and overall amount of reactant **500** are limited by the preference of having an excess of reactant **500** as well as maintaining the reactant's solubility in the available volume. Preferably, the concentration of the reactant **500** is a minimum of 1 mg/mL.

The reactant **500** may be applied to a working electrode with any method that allows for volume and position control capable with techniques such as screen printing, ink-jet printing, air brush, and pad printing. For example, for application methods that use a nozzle or a screen that must continuously pass solution, the reactant **500** is preferably applied without glutaraldehyde, and then, the glutaraldehyde is placed down. This avoids fouling the nozzle with solidifying material. Once applied, the reactant **500** is dried and cured with the times of each varying based upon the amount and thickness of reactant layer(s) and the composition. Suitable drying conditions include temperatures up to 150°C, controlled humidity, and cure times of 15 minutes to 24 hours.

The GOX enzyme will saturate at concentrations of approximately 3 mM glucose. In order to detect higher levels of glucose, the concentration reaching the reactant **500** must be held to a fraction of the total concentration. To accomplish this, a membrane **300** is disposed over or around the reactant **500**. In one preferred embodiment, the membrane **300** is disposed over or around all of the electrodes **400** as shown in **Figure 3** In another preferred embodiment, the membrane **300** is disposed over or around the working electrodes **410** and **420** as shown in **Figure 2**. Alternatively, the membrane **300** is disposed over or around each electrode **400** or each working electrode **410** and **420** as shown in **Figures 1**.

The membrane **300** preferably is a diffusion-limiting membrane that extends the linear range and the lifetime of the assay device **1000** system and makes it useful in a continuous/continual monitoring system. The membrane **300** has pores which regulate diffusion of an analyte therethrough. Therefore, the membrane **300** may be sized to limit the rate at which the analyte or an interferent makes contact with the reactant **500,** thereby increasing the linear range of the assay device **1000**. For example, the membrane **300** may have low porosity to reduce glucose flux. As such, the membrane **300** limits the amount of analyte that is present at the electrodes at any one time, allowing the electrodes **400** to operate continuously over long periods of time without depleting the reactant. In fact, a monitoring unit coupled to the at least one working electrode may continuously draw fluid through the assay device and detect the presence or level of an analyte in excess of 24 hours, more preferably in excess of 48 hours, and still more preferably in excess of 70 hours.

One preferred membrane **300** is a 0.01 μm pore diameter polycarbonate ("PC") track-etch member 6 μm thick. Other suitable membranes that effectively produce a diffusion rate include dialysis membranes, polyurethane membranes, or polyvinylchloride membranes. Castable membranes such as NAFION™,

cellulose acetate, silastics and alkoxy silanes are also effective for this use. Additionally, multiple membranes may be used.

The membrane **300** may be secured by a layer of cross-linked BSA in the same buffer as the reactant **500**. In one preferred embodiment, the cross-linked BSA consists of 60 mg/mL BSA dissolved in PBS containing 10% glycerol and 0.01% NaN3. In addition, 20 μL/mL of 25% glutaraldehyde may be added immediately before use.

Any effective amount of this layer of cross-linked BSA may be used. In one preferred embodiment, a 2 μL drop of the cross-linked BSA is placed on the reactant covered electrode. Then, a 5 mm diameter circle of the 0.01 μm PC membrane is placed over the drop of cross-linked BSA or other suitable, large polyamine. The membrane **300** is gently pressed into place under a sheet of parafilm. It is allowed to cure for 16 hours at room temperature under the parafilm, and then the parafilm is removed.

The membrane **300** may be disposed over or around the reactant **500** by any suitable method including lamination, gluing, pressing, rolling and stretching. Any such method should not be destroyed by the fluid to be collected and analyzed. However, if an additional component is added, such as glue or other adhesion material, the additional component should be permeable in the fluid, such as Nafion is permeable in an aqueous-based fluid. Other suitable adhesives include epoxies, UV curable adhesives, pressure sensitive adhesives and hydrogels such as HEMA.

In operation, the assay device **1000** is positioned on a tissue site overlying one or more openings made in the tissue. The openings in the tissue may be made by a variety of means, such as those disclosed in commonly assigned U.S. Patent No. 5,885,211. Fluid enters the assay device **1000** of **Figure 1** through the

inlet port **910**. Under application of vacuum at the outlet port **920**, the fluid travels through the well **250** and contacts the membrane **300**. The membrane **300** is permeable to the analyte in the fluid and thereby allows the analyte to contact the electrodes **400** and the reactant **500**. The analyte reacts with the reactant **500** to generate a reaction product. The reaction product contacts the working electrode **420** and creates electrons, thereby generating a current flow. The fluid continues through the outlet port **920** whereupon it exits the assay device **1000**. Current flow across the working electrode(s) is measured, and from this a measurement of the analyte is obtained. The assay device **1000** may be used in conjunction with amperometric and coulometric measuring techniques.

In an amperometric measurement the current (charge/second) is measured at the applied voltage. This can be measured continuously, which is a preferred method in a flowing system. With a coulometric technique, the total charge accumulated over a period of time is measured after a voltage is applied. Typically, the fluid is allowed to react with the reactant over a fixed period of time, thereby generating a reaction product. Then a voltage is applied and the current, which is measured over a fixed period of time, is integrated (added) to calculate the total amount of charge produced by the reaction product. This alternative method has the advantage of generating larger signals and reducing the impact of electroactive interfering substances.

The membrane **300** preserves the life of the reactant **500** by helping to hold the reactant **500** in place and to thereby reduce the risk of rapid dissolution of the reactant **500** in the fluid. Also, by restricting the amount of analyte and interferents to the reactant **500**, the membrane **300** helps ensure that the reactant **500** is in excess than what is needed to fully engage the analyte. In this way, as the reactant **500** degrades over time, it may remain in excess and deterioration in performance will be minimized.

Referring now to **Figure 2,** a variation of the assay device **1000** is shown in which a well **250** opens into an outlet port **920**. In such a configuration, the outlet port **920** does not provide fluid communication through the support base **600**, the adhesive layer **700**, and the top layer **800**. However, the outlet port **920** is suitable for connection to a supply of vacuum sufficient to draw fluid through the well **250**. The outlet port **920** may be filled with wiring and drain tubing, and then epoxy sealed.

Figure 3 shows another alternative preferred embodiment of the assay device **1000**. As in **Figure 2**, the well **250** is coupled to the outlet port **920**. However, **Figure 3** shows an assay device that comprises only a support base **600** and bottom layer **100.** The bottom layer **100** provides the well **250** to expose the membrane **300** and electrodes **400** to the fluid.

Figure 4 shows another embodiment of an assay device **1000** according to the present invention. This embodiment includes a bottom layer **100**, a channel-forming adhesive layer **200,** a support base **600**, an adhesive layer **700**, and a top layer **800**. **Figure 4** also includes an inlet port **910**, a well **250**, an outlet port **920**, a calibration port **950**, and drain tubing **940**. Although not shown, this embodiment includes at least one working electrode, a reference electrode, and a reactant proximate to the working electrode as shown in **Figures 1-3**. This embodiment may optionally include at least one membrane, as shown in **Figure 1**. The electrodes are connected to a monitoring unit **970** via leads **960**. The monitoring unit **970** is also connected to assay device **1000** via the drain tubing **940**. The drain tubing **940** provides vacuum to the assay device **1000**.

Similar to the assay device **1000** of **Figure 2,** the well **250** shown in **Figure 4** opens into the outlet port **920**. The calibration port **950** is suitable for connection to a reservoir **980** containing calibration fluid. In addition, the

calibration port **950** may include a membrane **990** permeable to the calibration fluid.

In one embodiment, the calibration fluid consists of water and the analyte to be detected. Other compounds may also be present, such as surfactants, which ensure smoother flow by reducing surface tension, such as SDS, or any of the detergents herein described. Additionally, preservatives such as azide, EDTA, or any antibacterial or appropriate biocide that will not degrade of interfere with the reactant's performance may be added to the calibration fluid. Moreover, the calibration fluid may include thickeners, such as polymers and proteins, to simulate the flow characteristics of the analyte-containing fluid that is being measured.

The reservoir **980** is in fluid communication with the well **250** such that the calibration fluid flushes the well **250** to contact the electrodes with the calibration fluid. The calibration fluid is removed from the well **250** through the outlet port **920** under application of vacuum.

The reservoir **980** may release the calibration fluid into the well **250** using any effective mechanism. In one embodiment, the reservoir **980** comprises a bag-like member that opens and releases the calibration fluid into the well **250** in response to application of vacuum applied at the outlet port **920**. Moreover, the reservoir **980** may be formed of a material that when mechanically punctured releases the calibration fluid into the well **250**.

Alternatively, the membrane **990** is a self-sealing membrane that is rupturable to allow introduction of the calibration fluid such as by a syringe containing calibration fluid to deliver the calibration fluid into the well **250**. Preferably, the calibration fluid is delivered into the well **250** while vacuum is applied at the outlet port **920**. In another preferred embodiment, the calibration

fluid is introduced into the well **250** via a valve that operates as a one-way valve or is controlled external to the assay device **1000**.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. An assay device for detecting an analyte in a fluid, comprising:
   a) an inlet port to receive fluid;
   b) a well in fluid communication with the inlet port;
   c) an outlet port in fluid communication with the well to discharge fluid;
   d) at least one first working electrode and at least one reference electrode disposed within the well;
   e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and
   f) at least one membrane disposed over or around the reactant to regulate contact of the analyte in the fluid with the reactant.

2. The assay device of claim 1, wherein the inlet port is suitable for alignment with holes/porations in tissue from which fluid is drawn.

3. The assay device of claim 1, wherein the outlet port is suitable for connection to a supply of vacuum sufficient to draw fluid through the well.

4. Thc assay device of claim 1, wherein the at least one first working electrode is comprised of a catalytic metal.

5. The assay device of claim 1, wherein the at least one first working electrode is comprised of platinum, palladium, chromium, ruthenium, rubidium, or mixtures thereof.

6. The assay device of claim 1, wherein the at least one reference electrode is comprised of silver/silver-chloride.

7. The assay device of claim 1, further comprising at least one counter-electrode disposed within the well.

8. The assay device of claim 1, further comprising a second working electrode disposed within the well.

9. The assay device of claim 1, further comprising at least one counter-electrode and a second working electrode disposed within the well.

10. A monitoring system comprising the assay device of claim 1, and further comprising a monitoring unit coupled to the assay device.

11. A monitoring system comprising the assay device of claim 1, and further comprising a monitoring unit coupled to the assay device, wherein the monitoring unit generates an analyte measurement from the assay device utilizing a coulometric or amperometric measurement technique.

12. The assay device of claim 1, wherein the quantity of reactant is disposed in or on the at least one working electrode.

13. The assay device of claim 1, wherein the quantity of reactant is comprised of glucose oxidase enzyme, glucose dehydrogenase, or mixtures thereof.

14. The assay device of claim 1, wherein the quantity of reactant is suitable to react with glucose.

15. The assay device of claim 1, wherein the at least one membrane comprises pores which are sized to limit the rate at which the analyte makes contact with the reactant.

16. The assay device of claim 1, wherein the at least one membrane comprises pores which are sized to limit the rate at which an interferent makes contact with the reactant.

17. The assay device of claim 1, wherein the at least one membrane is disposed over or around the at least one first working electrode.

18. The assay device of claim 1, wherein the at least one membrane is disposed over or around the at least one first working electrode and the at least one reference electrode.

19. The assay device of claim 1, further comprising a second working electrode disposed within the well and wherein the at least one membrane is disposed over or around the at least one first working electrode and the second working electrode.

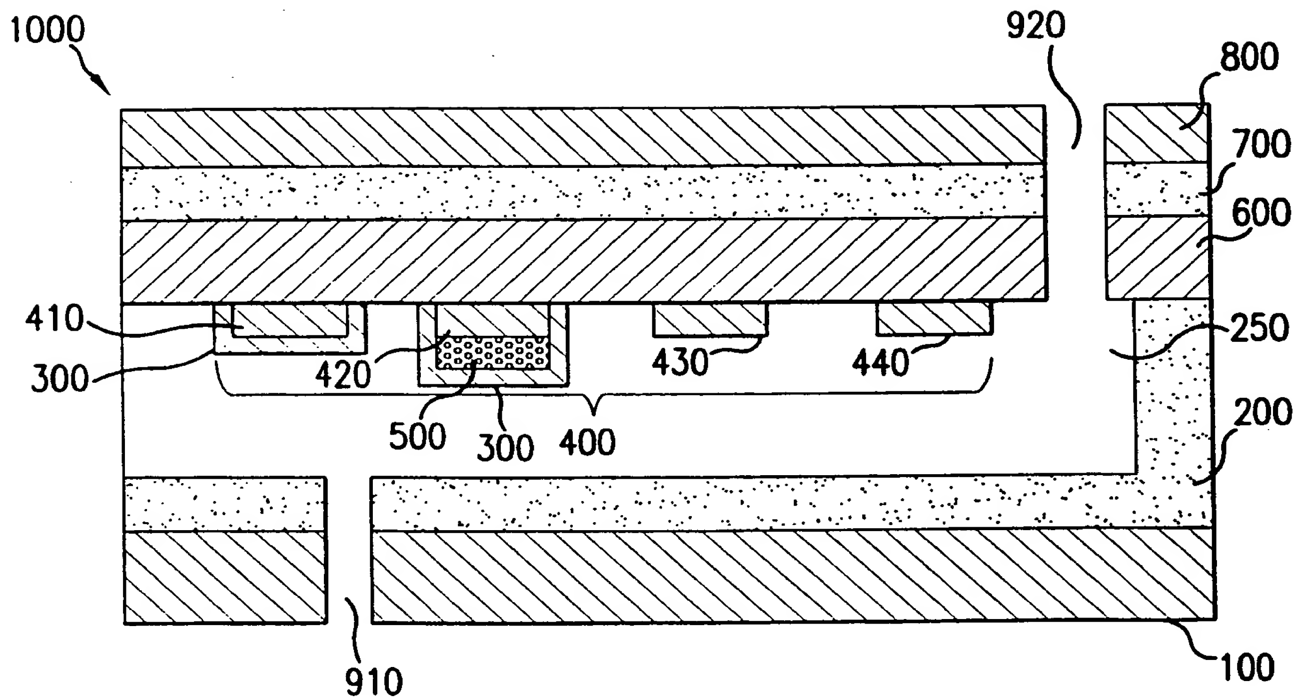
20. The assay device of claim 1, further comprising a reservoir containing a calibration fluid, wherein the reservoir is in fluid communication with the well such that the calibration fluid flushes the well and is removed from the well through the outlet port.

21. The assay device of claim 20, wherein the reservoir comprises a bag that opens and releases the calibration fluid into the well in response to application of vacuum thereto applied at the outlet port.

22. The assay device of claim 20, wherein the reservoir is formed of a material that when mechanically punctured releases the calibration fluid into the well.

23. The assay device of claim 20, further comprising a calibration port that couples the reservoir to the well, wherein the calibration port comprises a membrane permeable to the calibration fluid.

24. An assay device for detecting and enabling measurement of an analyte in a fluid, comprising:
   a) an inlet port to receive fluid,
   b) a well in fluid communication with the inlet port,
   c) an outlet port in fluid communication with the well to discharge fluid,
   d) at least one first working electrode and at least one reference electrode disposed within the well,
   e) a quantity of reactant that reacts with the analyte to form a reaction product, wherein the reaction product is in fluid communication with the at least one first working electrode; and
   f) a calibration port that is in fluid communication with the well.


1000
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800
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410
300
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430
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500
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910
100

FIG.1

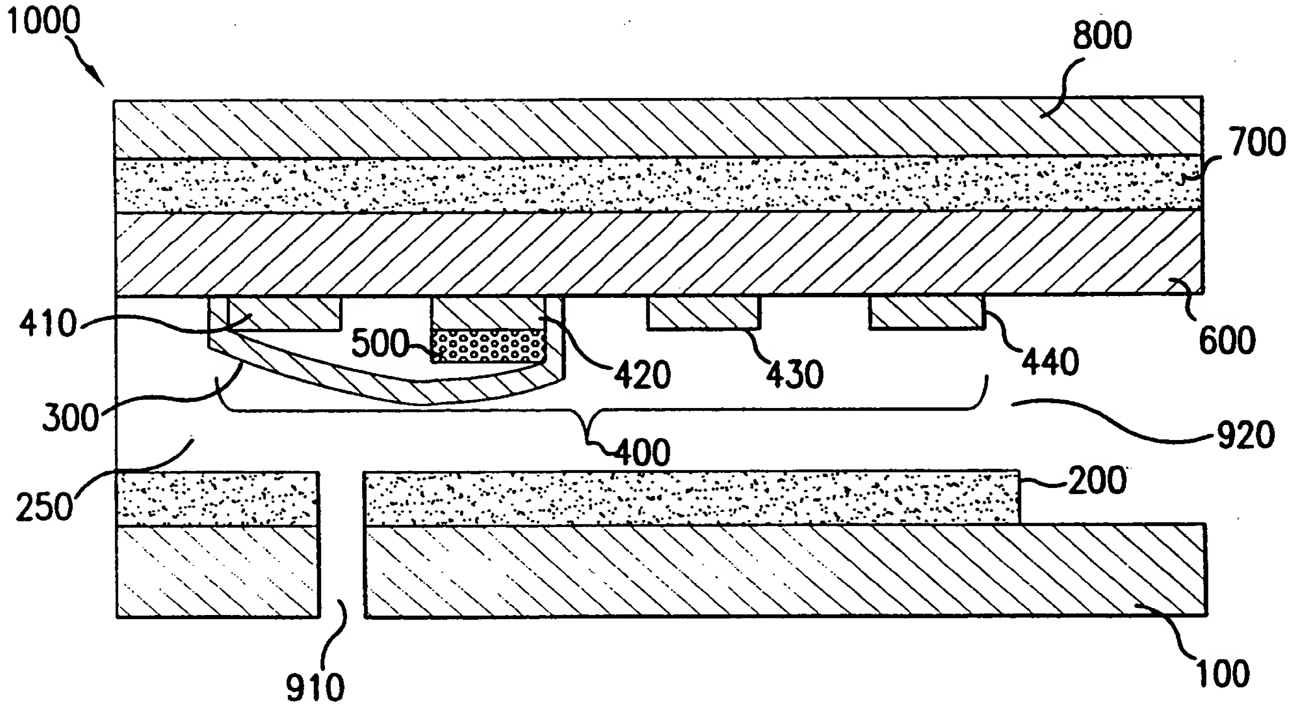

FIG.2

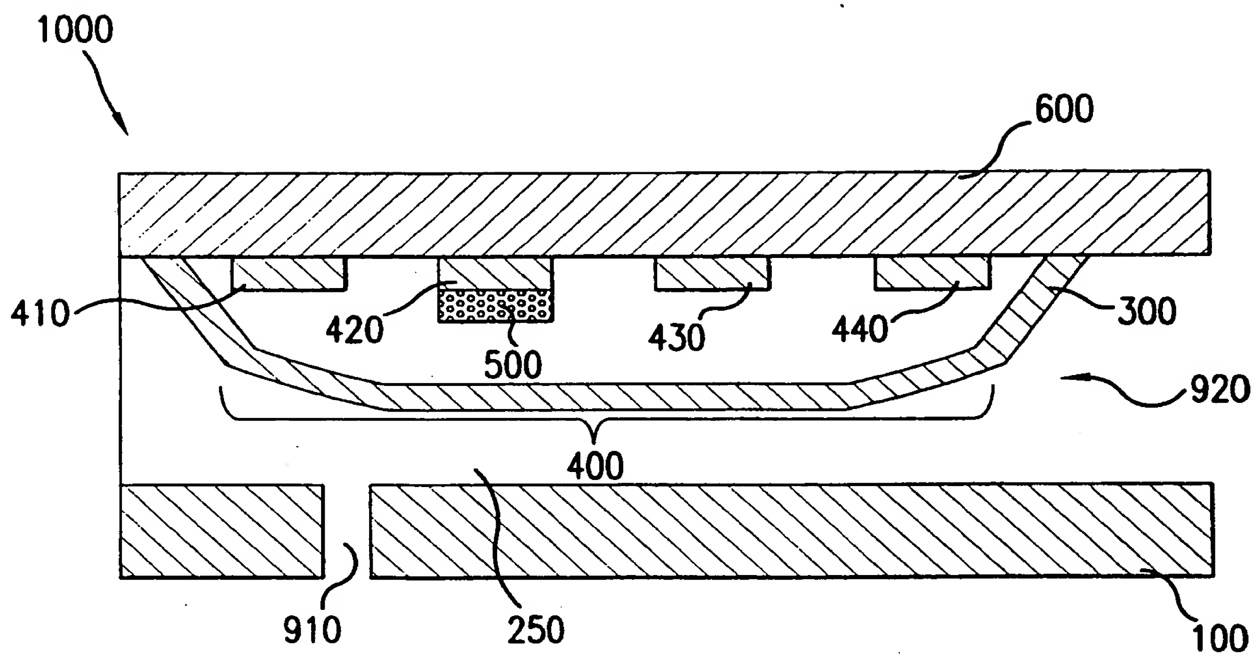

FIG.3

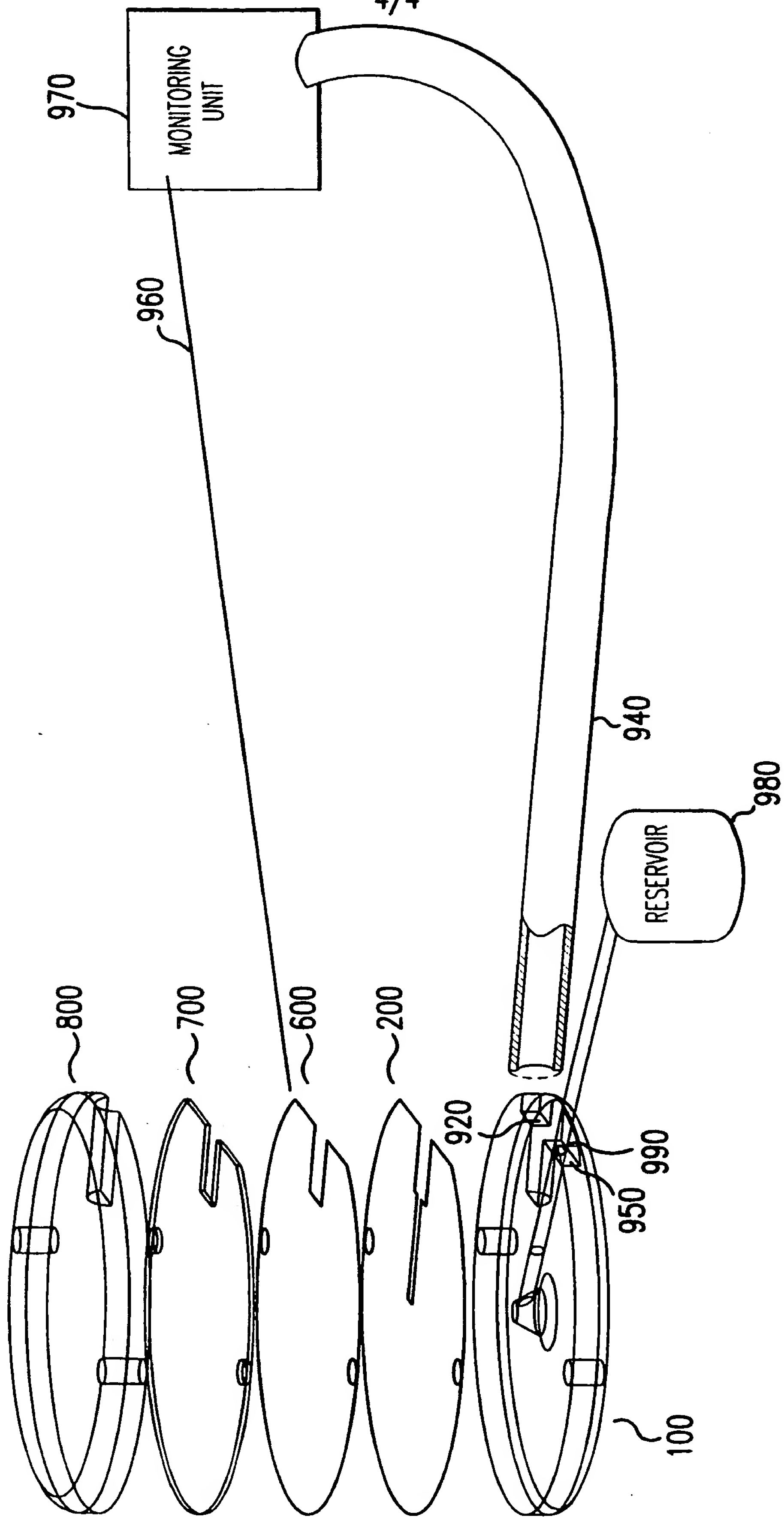
MONITORING UNIT
970
960
940
980
RESERVOIR
800
700
600
200
920
990
950
100

FIG.4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/09393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STEINKUHL R ET AL: "MICRODIALYSIS SYSTEM FOR CONTINUOUS GLUCOSE MONITORING" SENSORS AND ACTUATORS B,CH,ELSEVIER SEQUOIA S.A., LAUSANNE, vol. B33, no. 1/03, 1 July 1996 (1996-07-01), pages 19-24, XP000632919 ISSN: 0925-4005	1,4-6, 10-14,17
A	page 20, left-hand column, line 21 -page 21, left-hand column, line 43; table 1	2,15,16, 24

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2000	27/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Weihs, J

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/09393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HINTSCHE R ET AL: "CHEMICAL MICROSENSOR SYSTEMS FOR MEDICAL APPLICATIONS IN CATHETERS" SENSORS AND ACTUATORS B, CH, ELSEVIER SEQUOIA S.A., LAUSANNE, vol. B27, no. 1/03, PART 02, 1 June 1995 (1995-06-01), pages 471-473, XP000516364 ISSN: 0925-4005	1,3-6, 11-14,17
A	page 471, right-hand column, line 1 -page 473, left-hand column, line 2; tables 1,2	15,16,24
Y	EP 0 453 283 A (TEKNEKRON SENSOR DEV CORP) 23 October 1991 (1991-10-23) column 2, line 18 -column 4, line 22; tables 1-3	1-7, 10-18,24
Y	WO 98 30891 A (IMPLANTED BIOSYSTEMS INC) 16 July 1998 (1998-07-16)	1-7, 10-18,24
A	page 4, line 27 -page 12, line 2; tables 2-4	20-23

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/09393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 4227235 A	17-08-1992
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